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Assessment of royal jelly freshness by HILIC LC–MS determination of furosine

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ABSTRACT

Royal jelly (RJ) is a high added value product, which can be spoiled and may lose its health-promoting properties according to the storage temperature and duration. Furosine, a compound generated at the early stages of the Maillard reaction, is a good marker of the freshness of RJ which can be used to detect heat treatments or prolonged storage of RJ samples. The determination of furosine content of RJ has been developed and validated by LC–MS. This new analytical method has the advantages of being sensitive and rapid. Moreover it avoids the time-consuming and cost-intensive SPE purification step of the usual HPLC-UV method.

The influence of the storage temperature was studied on 55 French RJs. Ten of them were additionally analyzed just after harvest which exhibited furosine contents around 20.1 mg/100 g of protein. The furosine content remained stable when stored 9 months at −18 °C, whereas a slight increase was observed for a storage temperature of +2/+5 °C, with values increasing up to 61.3 mg/100 g of protein. The analysis of 27 commercial RJs, after 9 months of storage at +2/+5 °C, displayed higher furosine contents up to 219.9 mg/100 g of protein with a mean at 97.1 mg/100 g of protein. These results underline that furosine is a suitable marker for assessing the quality of storage conditions and freshness of RJ.

1. Introduction

Royal Jelly (RJ) is a natural product secreted by the hypopharyngeal and mandibular glands of worker bees. It is used to feed bee larvae up to three days, queen larvae up to five days and only the queen all her life. RJ contains many components such as water, sugars, amino acids, proteins, fatty acids and vitamins (Antinelli et al., 2002; Daniele et al., 2011; Wytrychowski et al., 2012). RJ exhibits lots of health-promoting properties such as antibacterial, antiviral, antioxidant or anti-inflammatory activities (Bogdanov, 2011; Kamakura et al., 2001). Its consumption as dietary supplement or in cosmetics is constantly increasing in France. As French production is about 1–2 tons per year, it is necessary to import large amounts of RJ, mainly from Asia (Daniele et al., 2011). Nevertheless, there is no international regulation concerning RJ harvest, production, trade and, in particular, conservation. This last step is very important because due to poor storage conditions, RJ may be spoiled or may lose its health-promoting properties (Kamakura et al., 2001).

Two modes of conservation are generally observed for RJ: in freezer at −18 °C in Asian countries and in fridge at +2/+5 °C in European countries. Several studies have compared the composition of RJ at both storage temperatures, but no significant differences have been observed after several months of storage (Chen and Chen, 1995; Liming et al., 2009). However it has been observed that RJ becomes readily darker and more viscous at ambient temperature (Antinelli et al., 2003; Chen and Chen, 1995; Kamakura et al., 2001). These changes occur due to the Maillard Reaction (MR), which involves amino acids and reducing carbohydrates. It is important to detect the MR from the early stages and furosine is generally chosen as freshness marker in food as it is generated in the early stages of MR. Indeed, at the beginning of the MR, it occurs metabolites called Amadori's compounds leading to furosine after an acid hydrolysis (Gökmen and Serpen, 2009). Thus, furosine is the most specific and earliest indicator of the extent of damage during storage. So, in milk, dairy products and processed foods, the furosine content is usually measured to follow their degradations (Del Castillo et al., 2002; Delgado et al., 1992; Resmini et al., 1990). Studies done on honey (Cárdenas-Ruiz et al., 2003; Sanz et al., 2003; Villamiel et al., 2001) indicate that furosine is present in all the samples analyzed and that it is a good marker of storage conditions. Moreover, it can be used to detect overheating during honey manufacture.

Nevertheless, there are only few studies on the furosine content in RJ (Marconi et al., 2002; Messia et al., 2005). The method

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described for milk and dairy product has been applied in these studies to analyze furosine in RJ originated from Italy, China and Taiwan. These studies demonstrate the reliability of furosine as a marker of freshness and storage conditions of RJ. The results are promising but these studies have been conducted on few samples only, so it is important to confirm their data and analyze additional samples from other origins. Moreover, studies often compare the storage at 4 °C and at ambient temperature, but the comparison of two modes of conservation usually practiced (i.e. at +2/+5 °C and −18 °C) has not been studied. Our work aims at acquiring first data on French RJs, based on the analysis of lots of samples and to compare the furosine contents of French production, stored at +2/+5 °C or at −18 °C, to those of RJs commercially available and generally imported.

Furosine is generally quantified by liquid chromatography with UV detection at 280 nm wavelength. A purification step by SPE (Solid Phase Extraction) is usually implemented and long elution times are common (Resmini et al., 1990; Marconi et al., 2002; Bosch et al., 2008). As the content of furosine in freshly harvested RJ is very low, our objective was to develop and validate a sensitive and rapid method using liquid chromatography (LC) coupled to mass spectrometry (MS). This analytical method has already been employed for quantification of furosine in orange juice and tomato product (Del Castillo et al., 2002). The original and new method developed in our work has been applied to the analysis of fifty-five French RJs and twenty-seven imported RJs. Influence of storage temperature on the furosine content of RJ is reported.

2. Materials and methods

2.1. Chemicals and materials

Acetonitrile, formic acid, hydrochloric acid and ammonium formate were of high purity (>98%) and were obtained from Sigma–Aldrich. The water used during the experiments was ultrapure water.

Furosine (purity 72.4%) was purchased from PolyPeptide Laboratories (Strasbourg, France). The furosine stock standard solution (10 ppm) was prepared in ultrapure water. Working standard solutions (0.10, 0.25, 0.50, 1.00 and 2.00 ppm) were prepared by appropriate dilutions of the furosine stock solution.

French RJs samples (55) were produced by French beekeepers belonging to the GPGR (Groupement des Producteurs de Gelée Royale). This implies the respect of a quality convention, concerning the production, harvest, conservation and trade of RJ. These freshly harvested samples were collected throughout France, in different areas during the 2012 harvest season. The samples were divided in two parts and stored in the dark at −18 °C or at +2/+5 °C.

Twenty-seven commercial RJs were purchased on Internet, from different suppliers between March and April 2012. These samples were imported but it was not mentioned any geographical origin nor any date of harvest on the labels. All the commercial samples were stored at +2/+5 °C, in fridge.

2.2. Protein content

The protein content was determined by multiplying the nitrogen content of the RJ by the multiplicative factor 6.25, corresponding to the reverse nitrogen content of a protein (Salo-Väänänen et al., 1996).

The nitrogen content was determined as described before (Wytrychowski et al., 2012). Here, an amount of 1.5 mg of RJ was weighed in a silver capsule and introduced in the combustion unit. Then, a dry combustion permitted to determinate the nitrogen content, according to the Dumas combustion method (norm NF ISO 13878).

2.3. Furosine content

2.3.1. Sample treatment

An aliquot of RJ sample (0.35 g), corresponding to about 30–70 mg of protein, was hydrolyzed into nitrogen with 8 mL of HCl 8 N at 110 °C for 23 h. The hydrolyzate was then filtered through 0.45 μm GHP membrane (Acrodisc®). This solution was diluted ten times in HCl 3 N before injection in HPLC–MS.

We have to note that a purification of the hydrolyzate by SPE (Solid Phase Extraction) is indispensable to decrease matrix interferences for the usual determination of furosine in royal jelly by HPLC-UV. Here it is not necessary as the MS detection is specific to furosine and as we have previously checked the absence of interferents from the matrix on the detection. Nevertheless we have compared the results obtained with and without the purification SPE on a Sep-Pak C18 cartridge (500 mg) from Waters (Waters Corp., Milford, MA). The cartridge was conditioned (5 mL methanol and 10 mL H_2O) and the hydrolyzate (0.5 mL) was added. The eluted liquid was discarded, and furosine was then eluted with 1 mL × 4 of HCL 3 N. The cartridge was dried in air and the eluates were collected in a 5 mL volumetric amber glass filled at 5 mL with HCl 3 N solution. The solution was filtered at 0.45 μM before injection. The results obtained with and without the purification step were quite equivalent and a small loss of furosine (< 2%) was observed during the purification. So this step was removed in our method.

2.3.2. Analytical conditions

The LC–MS analysis was carried out on an Agilent 1100 series. The chromatographic system was composed of a quaternary pump (G1311A), an injector (G1313A) and a diode array detector (DAD, G1315A) coupled to a quadrupole mass detector 1100 series LC/MSD used in the electrospray positive mode. The analytical separation was performed on a Nucleodur® Hilic column (125 mm × 2 mm; 3 μm; Macherey Nagel) maintained at 50 °C. Indeed, due to highly polar chemical functions (amino-acid), Hilic technology is well suited for separation technology in the case of furosine compound. The flow was constant and fixed at 0.3 mL/min. 2 μL of the sample solution were injected in the system. The mobile phases were composed of ammonium formate (0.01 M) in ultrapure water as mobile phase A (pH 6.5) and of acetonitrile with 0.1% formic acid as mobile phase B. The following gradient was used: start with 85% B during 1 min, then decrease to 50% of B at 6 min and stay 4 min. The total run time was about 10 min. The MS detection was performed in the selected ion monitoring mode (SIM) using target ion at m/z 255.1 and a fragmentor fixed at 60 V. The wavelength of the DAD was fixed at 279 nm.

The quantification of furosine was realized by external calibration. By convention, furosine content was expressed as the mass of furosine per 100 g of protein. Each sample was analyzed in triplicate.

2.3.3. Method validation

Several criteria were studied in order to validate the method developed: the linearity, sensitivity (by calculating detection and quantification limits), specificity, trueness and repeatability.

The linearity was determined by eight replicates analysis of six different concentrations over the range of calibration defined.

The limit of detection (LOD) was the lowest concentration of furosine that could be detected and the limit of quantification (LOQ) was the lowest concentration that could be quantified with an acceptable level. The LOD and LOQ were thus the concentrations for which the signal to noise ratio were 3 and 10, respectively, of